Studies on blood coagulation factor V

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I. The Interaction of Salts of Fatty Acids and Coagulation Factors

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It is common knowledge that the coagulation factors II, VII, IX and X are readily adsorbed onto a variety of insoluble anorganic powders such as $BaSO_4$, $Ca_3(PO_4)_2$, $Mg(OH)_2$, $Al(OH)_3$, etc. This adsorption is a well-known tool in blood coagulation research, and detailed studies of the phenomenon have been reported (Prydz 1964, Voss 1965).

It is less well known that some coagulation factors are adsorbed by salts of fatty acids. Vroman (1958) was among the first to observe this phenomenon, which he explained as the result of the interaction between the hydrophobic surface offered by the adsorbant and coagulation factors with a hydrophobic nature. He reported that factors I, V, VIII and thrombin were adsorbed by salts of fatty acids, and consequently considered these factors to be prone to hydrophobic interactions. Vroman also suggested that such interactions play an important role in the normal coagulation mechanism (Vroman 1964). Recent progress in the elucidation of the reaction mechanism of blood coagulation has re-affirmed the importance of this suggestion (Jobin 1966, Barton et al. 1967, Esnouf & Jobin 1967, Hemker et al. 1967 a & b).

We began the study on the influence of the salts of fatty acids on the coagulation factors with a double purpose. In the first place, we wanted to explore the possibilities of preparing specifically deficient plasmas by adsorption of certain factors and study the purification of coagulation factors by elution from hydrophobic powders. In the second place, we hoped that the system could be used as a model to further elucidate the role of hydrophobic interactions in the coagulation process. Although the exact nature of the interaction between procoagulant proteins and hydrophobic surfaces is not known, we will use the term *adsorption* throughout to indicate the phenomenon.

Part of the material in this article was presented at the Vth Congress of the Federation of European Biochemical Societies in Oslo, summer 1967 (Kahn & Hemker 1967).

Materials and Methods

The chemicals were obtained from the following sources: Hirudin: Sigma, St. Louis U.S.A.; Ba-Stearate: K & K Laboratories, New York U.S.A. Batches Nos. 45889 & 4152; other salts of fatty acids: Carl Roth O.G.H., Karlsruhe Western Germany.

All plasmas were made platelet free by centrifugation for 30 min at 20,000 g. When necessary they were stored at $-20°$ C in plastic tubes after centrifugation.

For the estimation of factors II, V, VII and X, and of VII and X in combination, one-stage estimations were carried out as described by van der Meer et al. (Van der Meer, 1968). Fibrinogen was determined according to Claus (1957). Factors VIII, IX and XII were estimated according to

Veltkamp et al. (1968). Factor XI was determined as was factor XII, but using congenitally factor XI-deficient plasma as a reagent instead of factor XII-deficient plasma.

All experiments were carried out at least in quadruplo, and all coagulation-factor determinations within one experiment were also carried out in quadruplo. The adsorption was carried out at room temperature.

Due to the hydrophobic nature of the adsorbants, it is difficult to mix them thoroughly with the plasma. To solve this problem, the adsorbants were suspended in the plasma by means of a Potter Elvehjem homogenizer consisting of a glass tube and a mechanically driven teflon pestle.

The powder was separated from the plasma by centrifugation for 10 min at 15,000 g at 4° C, but the supernatant was not entirely free from floating particles and heavier centrifugation did not improve the separation. Filtration was therefore applied to free the supernatant of these particles (Paper n. 5161 14, Macherey Nagel & Co. Duren Germany); the result was a perfectly clear plasma.

All calculations of adsorbed amounts are based on comparison of the adsorbed plasma with the same plasma that underwent exactly the same treatment (e.g. homogenizing in the Potter Elvehjem tube, centrifugation, filtration, dialysis, changes in pH and temperature etc.) for exactly the same lapse of time except for the admixture of the stearate.

When different anticoagulants were compared, the blood always came from the same donor at the same time, the only difference being the anticoagulant used.

Dialysis of plasma samples was carried out for two times 12 hrs at 4° C against 200 times the volume of 0.15 M NaCl, in 0.05 M Tris-HCl buffer (pH 7.4).

Gel filtration of plasma samples was carried out on Sephadex G-25-fine in a 2.5 × 40 cm column with 0.05 M Tris-HCl buffer (pH 7.4) in 0.9% saline as eluent. The eluate was continuously screened for U.V. adsorbancy with a recording Ultraviolet adsorbtiometer (L.K.B. Uvicord 4700 with recorder 6520 H).

Experimental Results

The adsorption of various coagulation factors from human plasma onto Ba-Stearate is shown in Table 1. Factors V and XI are the most readily adsorbed, but factor X is adsorbed almost as easily. Factors XII and I take an intermediate position, and the adsorption of factors II, VIII and IX and probably also that of factor VII, is not very marked. The results of a comparable experiment with bovine plasma are shown in Table 2. In this plasma the difference between the various factors are less marked than in human plasma.

Table 3 shows the influence of pretreatment of the stearate with albumin or fibrinogen. The adsorption of coagulation factors onto the now *wettable* powder shows little alteration due to this procedure.

Table 1. Adsorption of the Clotting Factors of Human Citrated Plasma onto Ba-Stearate.

Conc. Ba-Stearate (mg/ml)	Percentage adsorbed								
	F.I	F.II	F.V.	F.VII/X	F.X	F.VIII	F.IX	F.XI	F.XII
2	12	0	0	25	—	—	—	—	—
5	18	0	0	31	—	—	—	—	—
25	19	4	88	12	70	0	0	82	0
50	18	21	94	55	68	0	0	—	0.5
75	33	40	94	61	—	—	—	96	15
100	52	40	95	68	84	—	—	—	50
150	48	34	100	70	96	20	13	—	55
200	57	30	100	78	98	—	—	100	75

0: indicates no adsorption observed, — : indicates no experiment done.

Table 2. Adsorption of the Clotting Factors of Bovine Plasma onto Ba-Stearate.

Conc. Ba-Stearate (mg/ml	Percentage adsorbed									
	F.I		F.II		F.V		F.VII/X		F.XII	
	C	O	C	O	C	O	C	O	C	O
2	13	0	0	—	35	—	0	—	25	—
5	0	0	2	–8	15	–32	–20	0	60	—
25	13	0	31	31	10	35	15	35	61	—
50	10	1	39	60	30	30	25	62	65	—
100	29	16	43	62	25	35	30	77	71	—
150	43	40	50	63	64	70	25	82	78	—
200	48	49	73	64	78	77	73	88	85	—
250	61	57	73	73	82	84	88	84	88	—

C: citrated plasma; O: oxalated plasma. —: no experiment done; 0: no adsorption observed. Negative values indicate that a phenomenon of activation has been observed, i.e. that the activity of the plasma was higher after adsorption than before.

Table 3. Influence of Albumin or Fibrinogen on the Adsorption of Coagulation Factors by Ba-Stearate.

Pretreatment of Ba-Stearate	Protein content Ba-Stearate (% W/W)	Percentage adsorbed			
		II	V	VII/X	total protein
Buffer	—	54	98	78	34
Buffer + 5 mg/ml albumin	5	46	98	77	16
Buffer + 5 mg/ml fibrinogen	10	60	90	60	20

The Ba-Stearate was pretreated by mixing 2 g with 50 ml Tris-HCl buffer (pH 7.5) 0.02 M with admixtures as indicated. After centrifugation (20 min; 20,000 g; 4° C), the pellet was resuspended in the same buffer without admixtures and washed twice. Then the pellet was mixed with the plasma to a final concentration of 200 mg of stearate per ml.

In this table also the total amount of protein adsorbed is indicated. It shows a general phenomenon i.e. that from plasma about 1,2 mg protein is adsorbed per 10 mg Ba-Stearate added. Under the conditions tested (i.e. the conditions shown to be varied in different tables) this figure was remarkably constant. About half of this protein could be eluted by 0.5 M phosphate pH 7.0.

Table 4 shows the influence of the anticoagulant used in preparing the plasma. In human plasma the strong adsorption of factor V is independent of the anticoagulant used, as is the adsorption of fibrinogen. The adsorption of factor II is more marked in oxalated plasma than in citrated plasma. The adsorption of factors VII and X is influenced to a lesser degree. In the same table it is again seen that bovine factors, and especially bovine factor V, are adsorbed less readily than human factors. Factor X from human serum appears to be readily adsorbed.

The differences resulting from the use of various anticoagulants suggest that slight variations in the concentration of Ca-ions might influence the adsorption characteristics. This was checked experimentally by depriving plasmas of Ca^{++} ions by dialysis

Table 4. Influence of the Anticoagulant on the Adsorption by Ba-Stearate.

Material	Anticoagulant	Final conc. (mM)	Percentage adsorbed					
			F.I	F.II	F.V	F.VII/X	F.X	F.VII
Human plasma	Na-citrate	10	58	25	98	80	85	30
Human plasma	Na EDTA	18	47	55	95	85	—	—
Human plasma	Na-oxalate	18	54	75	58	94	98	27
Bovine plasma	Na-citrate	10	29	39	19	25	—	—
Bovine plasma	Na-oxalate	18	16	62	35	77	—	—
Human serum	—	—	—	—	—	93	90	55

The concentration of Ba-Stearate used was 100 mg/ml.

or gel filtration, adding known amounts of Ca, and then carrying out the adsorption experiments immediately.

Table 5 shows that Ca^{++} ions enhance the adsorption of factors II, VII and X, but do not influence the adsorption of factor V. In the presence of Ca^{++} ions no coagulation was observed. Because thrombin is easily adsorbed onto Ba-Stearate (Table 6) and

Table 5. Influence of Ca^{++} Ion Concentration on the Adsorption by Ba-Stearate.

Material				Percentage adsorbed													
				Factor II					Factor V					Factor VII/X			
				0	5	25	50	100	0	5	25	50	100	0	5	25	50	100
P	B	O	G	71	98	96	98	98	97	84	98	99	99	28	38	64	75	83
P	B	C	G	86	95	98	99	92	97	92	98	99	99	60	77	88	80	84
S	B	–	G	—	—	—	—	—	94	98	94	99	99	54	55	92	85	88
P	B	O	D	60	93	97	98	97	93	98	99	97	99	45	60	83	89	81
P	B	C	D	88	94	97	98	99	97	96	99	99	99	57	87	85	90	75
S	B	–	D	—	—	—	—	—	84	94	97	99	99	66	78	81	92	93
P	H	C	G	85	90	92	98	91	98	98	98	99	99	76	82	92	91	87

P: plasma
H: human material
O: oxalated plasma
G: decalcification by gel filtration
D: decalcification by dialysis
S: serum
B: bovine material
C: citrated plasma

The figures at the top of the columns indicate the final concentration of Ca^{++} in mM; 150 mg/ml of Ba-Stearate was used throughout.

Table 6. Adsorption of Thrombin by Stearates.

Final concentration of stearate (mg/ml)	Percentages adsorbed	
	Ba-Stearate	Na-Stearate
2	94	95
5	97	96
10	98	98
25	99	98
50	100	100
100	100	100

Table 7. Influence of Ca^{++}Ion Concentration, Ba-Stearate Concentration, and Hirudin on the Adsorption by Ba-Stearate.

Concentration Ba-Stearate (mg/ml)	Hirudin	Percentage adsorbed																			
		Factor II					Factor V					Factor VII/X					Factor I				
		0	5	25	50	100	0	5	25	50	100	0	5	25	50	100	0	5	25	50	100
5	−	*−47*	*−200*	*−88*	*−280*	*−270*	*−30*	*−107*	*−442*	*−58*	*−69*	*−2*	*−50*	*−9*	*−16*	*−46*	92	99	90	90	90
	+	21	*−25*	0	16	*−9*	50	63	80	74	77	34	27	18	25	27	60	60	62	60	92
50	−	35	23	66	85	88	50	*−17*	50	78	23	22	55	34	55	53	92	90	75	90	90
	+	65	87	86	84	90	84	94	93	86	73	56	63	86	87	70	61	80	83	95	95
100	−	67	40	90	92	96	90	70	96	99	97	60	66	49	61	69	82	99	90	90	90
	+	66	58	80	86	81	91	80	84	96	92	60	58	61	56	62	66	78	78	96	90
150	−	86	95	96	98	99	97	92	98	99	99	60	77	88	80	84	99	99	99	99	99
	+	84	90	98	99	96	98	90	93	97	92	60	68	71	76	79	68	72	79	86	84

The final concentration of hirudin, if present, was 30 μg/ml. The figures at the top of the columns represent the final concentration of Ca^{++}ions. The material used was citrated bovine plasma rendered Ca^{++}-free by gel filtration on Sephadex G-25. Negative values (italics) indicate that the activity in the final supernatant was higher than the original activity.

When no hirudin was present during the adsorption, it was added to the plasma after adsorption in order to obtain comparable results.

subsequently inactivated (Vroman 1967), it might have left the reaction mixture before giving rise to observable clotting. Therefore the possibility cannot be excluded that activation phenomena occurring on the surface of the Ba-Stearate influence the activities found in the supernatant. Comparison of experiments carried out in the presence and absence of the selective thrombin inhibitor hirudin (Table 7) indeed shows that our findings are influenced by the occurrence of thrombin. This is especially evident at low concentrations of Ba-Stearate, where activation can easily gain over adsorption. As a rule, less adsorption is seen in the absence of hirudin, but of course this observation can be attributed to the presence of thrombin simulating specific clotting factors. Fibrinogen is better adsorbed in the absence of hirudin, which suggests that the fibrin monomer is more readily adsorbed than the fibrinogen molecule.

From these experiments it is also evident that the concentration of Ca^{++} ions has no significant influence on the adsorption of factors V and X onto Ba-Stearate, which forms an important difference between this adsorption and the adsorption onto phospholipid (Esnouf & Jobin 1967).

The influence of pH is shown in Fig. 1. Presumably, the changes in tertiary structure and/or other changes of the clotting factors brought about by a change in pH, strongly influence the adsorption characteristics. It must be kept in mind that the differences found indicate real differences in adsorption and not in inactivation at extreme pH's, because the adsorption was calculated with reference to a control that was kept at the same pH for the same lapse of time.

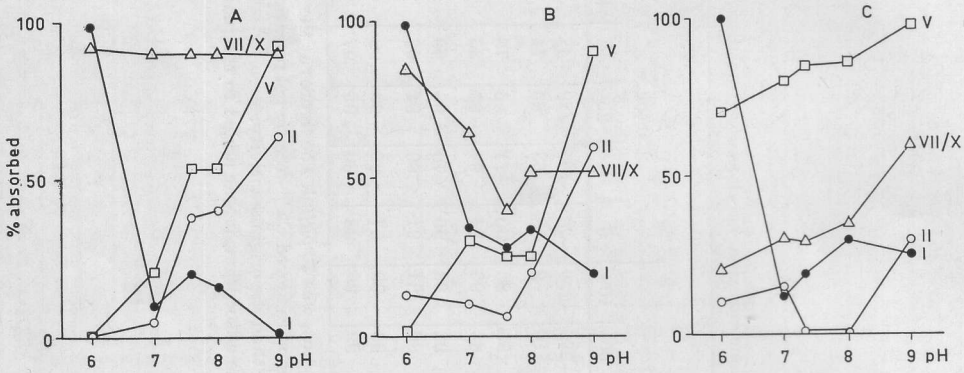

Fig. 1. The influence of pH on the adsorption of coagulation factors by Ba-Stearate. A. Bovine oxalated plasma 100 mg stearate/ml, B. Bovine citrate plasma 100 mg stearate/ml, C. Human citrate plasma 50 mg stearate/ml.

The effect of adsorption-time is shown in Fig. 2. The adsorption is virtually complete in 5 min. It can be seen from Table 8 that adsorption is favoured by elevation of the temperature. No fundamental differences seem to occur when stearates with different cations are tested (Table 9). As a rule, Pb-Stearate is inferior to the other stearates as an adsorbant, and Na-Stearate appears to be the best adsorbant. It is probable that the observed differences are dependent upon the physical state of the powder rather than upon the variation of the cation.

The differences between bovine and human plasma as observed with the use of Ba-Stearate are encountered with other stearates as well. Since it has been described that Na-Stearate can give rise to contact activation (Nossel 1964) the possibility had

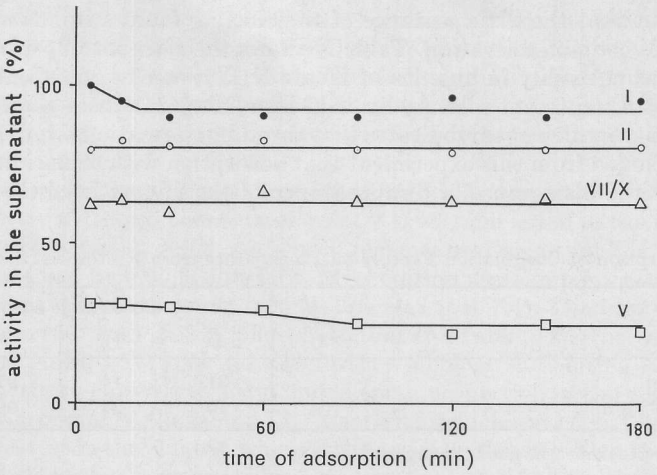

Fig. 2. Influence of incubation time on the adsorption of coagulation factors by Ba-Stearate.

Table 8. Influence of Temperature on Adsorption by Ba-Stearate.

Temperature °C	Factor I	Faktor II	Faktor V	Faktor VII/X
4	<1	<1	50	<1
10	<1	<1	45	<1
20	1	<1	49	<1
37	20	10	85	28

The material was human citrated plasma, the final concentration of Ba-Stearate 50 mg/ml.
The adsorption was carried out during 10 min.
The figures indicate the amount adsorbed.

Table 9. Influence of the Cation on Adsorption by Stearates.

Cation	Conc. mg/ml	Percentage adsorbed						
		I	II	V	VII	X	VII/X	XII
Ba	5	8	2	–4	–2	34	–9	0
	50	18	21	94	30	70	55	0
	100	55	40	93	50	88	—	—
	150	60	35	99	45	84	70	55
Na	5	33	17	91	17	42	38	74
	50	99	28	96	53	84	91	77
	100	99	69	99	90	98	—	—
	150	99	85	99	82	99	95	80
NH_4	100	70	55	99	93	99	—	—
Ca	100	13	50	99	20	95	—	—
Mg	100	55	40	99	30	89	—	—
Zn	100	75	75	99	93	99	—	—
Pb	100	0	20	75	10	50	—	—
Al	100	65	78	99	58	95	—	—

The material used was human citrated plasma.

to be ruled out that the disappearance of the clotting factors from the supernatant was a result of contact activation. Table X shows the relevant experiment. Because adsorption occurs readily in absence of factor XII, it can be ruled out that surface contact plays an important role. Addition of hirudin again causes quantitative changes in adsorption, which again indicates that thrombin formation occurs. Nevertheless it can be concluded from this experiment that adsorption with exclusion of activation phenomena is the main cause of disappearance of the factors from the supernatant.

Table 10. Adsorption of Coagulation Factors on Ba-Stearate from Factor XII Deficient Plasma.

	Percentage adsorbed			
	I	II	V	VII/X
Without hirudin	31	14	97	58
With hirudin 30 μg/ml	10	24	99	79

50 mg/ml Ba-Stearate was used throughout.

It has been reported that the bovine factor V activity taken from the plasma can be found again on the stearate powder used as an adsorbant (Vroman 1958). To study this phenomenon we did a series of experiments in which we adsorbed the plasma with stearate, washed the powder twice with Michaelis buffer, suspended the washed powder in Michaelis buffer (pH 7.4) and estimated the activity of the suspension. No appreciable activity was found in the washings. The results are shown in Table 11. It is seen that the activity found on the powder is always a small fraction of the activity that disappeared from the plasma. The factor V activity of the powder is relatively high.

Table 11. Activity of Stearate Powders on which Coagulation Factors have been Adsorbed

Material	Hirudin	Factor I		Factor II		Factor VII/X		Factor V		Factor XII	
	μg/ml	O	C	O	C	O	C	O	C	O	C
Normal human	0	<0.1	52.	0.3	37.	0.7	69.	4.5	93.	1.0	48.
Normal human	10	<0.1	33.	1.4	38.	1.1	72.	5.0	90.	1.4	43.
Normal bovine	0	<0.1	34.	0.4	47.	0.3	31.	18.0	29.	8.0	64.
Normal bovine	10	<0.1	28.	0.8	39.	0.3	28.	15.0	33.	10.0	69.
Factor XII-def.	0	<0.1	48.	0.7	41.	0.8	57.	15.0	95.	—	—
Factor XII-def.	10	<0.1	38.	1.3	43.	0.3	57.	6.0	97.	—	—

O = activity observed on the resuspended powder

C = activity calculated from the difference between original material and residual supernatant

The powder was resuspended in Michaelis buffer in a volume equal to the original volume of plasma. The activity is expressed as a percentage of the activity of the original plasma.

Means of 8 experiments

Citrated plasma was used throughout; essentially the same results were seen with oxalated plasma. The factor XII-deficient plasma came from a patient with congenital factor XII content of less than 2%.

100 mg/ml Ba-Stearate was used as an adsorbant. All experiments were also carried out with 5, 50, and 150 mg/ml, which gave no essentially different results. All experiments were duplicated with Na-Stearate substituted for Ba-Stearate; this also gave no different results. Treatment of the plasmas with celite (25 mg/ml) or dialysis against 0.05 M Tris HCl (pH 7.4) again made no difference.

This activity was found only if the stearate had been in contact with a plasma having factor V activity. No activity was observed on the powder that had not been in contact with such a plasma, and therefore the possibility of an aspecific influence on our factor V test could be excluded. No conditions could be found under which activities were detected on the powder that were significantly higher than those reported in Table 11.

We tried to elute the activities from the powder in many different ways. At pH 6.0, 7.0, and 8.0, we tested concentrations of 0.1, 0.3, and 0.5 M of the following salts: $MgCl_2$, Na-citrate, $CaCl_2$, NaCl, and Na-K phosphate. Except for the phosphate, the pH was maintained by 0.05 M Tris HCl. After elution, the sample was centrifuged and the supernatant dialysed against 0.05 M Tris HCl (pH 7.4). The best elution of activity was observed with 0.5 M phosphate buffer (Table 12). Increasing the ionic strength with NaCl 0.5–1.0 M did not further increase the eluting capacity of this buffer. The activity eluted was again only a small proportion of the activity lost from the plasma. Surprisingly, the factor VII/X activity, which was negligible on the powder itself, was greatest in the eluates, whereas the activity of factor V on the powder could not be eluted. This phenomenon is illustrated by Table XIII which shows the activities of both powder and eluate found in parallel experiments under exactly comparable conditions.

Table 12. Elution of Coagulation Factors from Ba-Stearate.

pH	Anticoagulant	Activity eluted (%)		
		Factor II	Factor VII/X	Factor V
6.0	oxalate	0.2	20	2.8
7.0	oxalate	0.4	30	0.8
8.0	oxalate	0.3	26	<0.1
6.0	citrate	0.2	18	1.5
7.0	citrate	0.2	18	0.6
8.0	citrate	0.6	26	0.2

Bovine citrated plasma. Elution with 0.5 M Phosphate buffer.

Discussion

From the experimental results presented it may be concluded that contact with stearates specifically lowers the concentration of some clotting factors from both human and bovine plasma (Tables 1, 2).

The disappearance is proportional to the amount of stearate added (Tables 1, 2).

One can imagine two different explanations for this phenomenon:

a) Coagulation factors are adsorbed onto the surface of the hydrophobic powder.

b) The hydrophobic surface catalyses a partial coagulation reaction causing the alteration and consumption of some factors. These two explanations are not mutually exclusive and a combination of the two may represent the real situation. Before trying to find out whether both explanations are valid, we will discuss the results in terms of adsorption. It is seen that from human citrated plasma, factor V and factor XI are most readily adsorbed, followed closely by factor X. Factors I and XII take an intermediate position, and factors II and VII are adsorbed least. From bovine plasma factors II, VII, and X are best adsorbed, and there is no great difference between

Table 13. Coagulation Factor Activity on Stearate Powders Compared to Activity Eluted from the Powders.

Origin of the plasma	Hirudin µg/ml	Ba-Stearate mg/ml	Factor II		Factor V		Factor VII/X		Factor XII		Factor VII		Factor X	
			P	E	P	E	P	E	P	E	P	E	P	E
Bovine	0	135	0.4	0.2	18	1.5	2	18	21	9	–	–	–	–
Bovine	30	135	0.8	1.3	15	0.4	3	8	12	2	–	–	–	–
Human	0	100	0.3	0.1	3.5	<0.1	0.6	1.8	1.6	0.6	–	–	–	–
Human	30	100	1.4	1.1	2.0	0.1	0.4	1.3	2.0	1.1	–	–	–	–
Human	0	50	0.1	2.2	4.9	<0.1	0.2	17	–	–	0.5	25	0.7	15
Human	30	50	0.3	3.0	3.5	<0.1	2.5	8.5	–	–	0.8	8.0	0.5	18
F. XII-def.	0	100	0.8	<0.1	3.3	<0.1	2.7	0.6	–	–	–	–	–	–

P = activity on the powder when suspended in the original volume of the plasma used, expressed as a percentage of the activity of the original plasma.

E = activity eluted from the powder expressed as a percentage of the activity of the original plasma.
The powder was eluted with 0.5 M phosphate (pH 7); the eluate was dialysed against 0.05 M Tris HCl (pH 7.4) and the volume reconstituted to the volume of the original plasma sample.

Each figure gives the percentage found as the mean of 5 experiments. Citrated plasma was used throughout.

these factors and factor V. The adsorption is most marked from oxalated plasma. From human oxalated plasma there is also a marked adsorption of factor II (Table 4). These results only partially confirm those of Vroman (1958, 1964) who reported factors I, V, VIII, and XI to be taken away by hydrophobic surfaces.

The adsorption seems to be due to a relatively specific interaction between the stearate and the clotting factors, because the stearate with an excess of proteins such as fibrinogen or albumin does not significantly influence the adsorption (Table 3). Moreover changes of Ca ion concentration, hirudin, anticoagulant or the source of the plasma (human or bovine) which did have an influence on the extent of the adsorption of the factors did not significantly alter the overall adsorption of proteins.

The general pattern of adsorption of the various factors is not changed when the cation of the stearate is changed. Marked differences can be found between various stearates, but these are probably due to the physical rather than the chemical properties of the powders. It was observed that the powders that were most difficult to mix with watery solutions were the most effective as adsorbants. Na-Stearate, which is a very good adsorbant, for instance, could hardly be mixed with the plasma.

As already indicated, the results can be explained by assuming that the hydrophobic surface partially catalyses a coagulation reaction, and that in this reaction some factors are chemically altered – either inactivated directly or adsorbed after alteration. The feasability of this explanation is at once clear when it is observed that under some conditions the plasma shows a higher activity after treatment with Ba-Stearate than before; a phenomenon which cannot be explained without the assumption that activation reactions play a role (Table 7). The fact that under these circumstances no complete coagulation is seen be can ascribed to the strong adsorption of thrombin by Ba-Stearate (Table 6). That thrombin does play a role in the process is readily seen from the influence of hirudin. The influence of Ca^{++}, which is not very marked at higher concentration of stearate (Table 5) but which can be seen at lower concentrations, is largely cancelled by hirudin (Table 8). Because hirudin is a specific inhibitor of thrombin (Markward 1958) this indicates that Ca^{++} acts via an activation of prothrombin. The action of Ca^{++} on the adsorption *per se* is a slight enhancement of the adsorption of all factors. This differs fundamentally from the situation with phospholipid, where Ca^{++} ions are necessary for adsorption of factor IX_a and X_a but inhibit the adsorption of factors V and VIII (Jobin 1966, Hemker & Kahn 1967).

Although a partial coagulation reaction must be assumed, we are of the opinion that the main action of stearates at a concentration of 50 mg/ml or higher is an adsorptive one, because:

a) The phenomenon is virtually instantaneous (Fig. 2).

b) The phenomenon has a pH dependency that does not show the same pH dependency as does the coagulation process, but rather suggests that drastic alterations of the tertiary structure of the coagulation factor molecules influence the adsorption, since it tends to extremes at extreme pHs (Fig. 1).

c) When factor V is removed from the supernatant by a coagulation process only it would be expected that a gradual increase was observed with rise of temperature (about a doubling of the rate with 10° C rise of temperature). Changes in temperature have only a slight influence on a mere adsorption (Zittle 1952) although the rate at which an adsorption equilibrium is reached slows down. The data from Table 8 again suggest that both adsorption and consumption of factor V occur, but that only at 37° C the consumption by coagulation plays a relatively important role. The observation on the factors I, II and VII/X do not contradict this view.

d) Hirudin does not change the general aspects of the adsorption.

e) The activation reactions triggered by contact activation do not seem to play an important role, since the adsorption from factor XII-deficient plasma in essentially the same as that from normal plasma.

From the experiments in which we tried to estimate the activity on the powder and in eluates from the powder it is clear that hydrophobic adsorption induces great losses of activity. The fact that only factor V shows some activity when adsorbed onto Ba-Stearate can be explained by the assumption that factor V maintains the structure in which it is physiologically active and exposes its active site when adsorbed onto a hydrophobic surface. Other parts of the molecule seem, however, to be deeply affected by the binding to the hydrophobic surface, because it is impossible to elute an active molecule in any quantity. The situation might well be comparable to the adsorption of factor V onto phospholipid in the formation of prothrombinase, where the phospholipid offers a hydrophobic surface on which factor V can adsorb with exposure of its active site. The fact that an excess of Ca^{++} ions hinders the adsorption of factor V onto phospholipid but not that onto Ba-Stearate, indicates that the two situations are not directly comparable however. The effect of excess Ca^{++} on the interaction of phospholipid and factor V may be due to an effect of Ca^{++} not on the factor V molecule, but on the phospholipid surface.

The adsorption of factor X_a onto stearates is probably not comparable to its adsorption onto phospholipid. In the first place, Ca^{++} ions are necessary for the binding of factor X to phospholipid (Jobin 1966, Esnouf and Jobin 1967, Hemker & Kahn 1967), which they are not for the adsorption onto stearates. In the second place, factor X does not expose its active site when adsorbed onto stearate, but it is not denatured since it is possible to elute the activity (Table 13).

We must conclude from these results that the part of the factor X_a molecule that bears the active site is the same as the hydrophobic site that binds to stearates. It might be argued that we are here studying the adsorption of factor X rather than the adsorption of factor X_a. With serum, however, essentially the same results are found (Table 4 and 5). Moreover, the spontaneous activation on the stearate surface in the absence of hirudin seems to be sufficiently important to imply the presence of quite appreciable amounts of factor X_a even when the adsorption is done from plasma.

Summary

The interaction between stearates and coagulation factors consists of adsorption of the factors and partial activation resulting in thrombin formation. Factors V and XI are most readily adsorbed, closely followed by factor X.

Activation is seen most clearly with low concentrations of the adsorbant. It can be inhibited by omission of Ca^{++} ions and addition of hirudin, thus it involves most probably the generation of thrombin. No thrombin is found in the reaction mixture due to the strong adsorption of thrombin on stearates. The effects of activation are small compared to the effect of adsorption time, pH, temperature and hirudin.

Activity of factor V can be demonstrated in the adsorbed state but the factor cannot be eluted. Factor X shows no activity when adsorbed, but can be eluted from the powder. This suggests that the biological activity of factor V resides in a hydrophylic part of the molecule, whereas the active centre of factor X probably is hidden but not destroyed by hydrophobic surfaces. The implications of these findings for the concept of prothrombinase formation on phospholipids are discussed.

Résumé

L'interaction entre les stéarates et les facteurs de coagulation consiste en une adsorption des facteurs avec activation partielle causant la formation de thrombine. Les facteurs V et XI sont le plus failement adsorbés, suivis de près par le facteur X.

L'activation est observée de la façon la plus nette avec les faibles concentrations de l'adsorbant. Elle peut être inhibée par l'omission des ions calciques et l'addition d'hirudine. Par conséquent la formation de thrombine est probablement impliquée dans ce processus. On ne trouve pas de thrombine dans le mélange de réaction à cause de sa forte adsorption aux stéarates. Les effets d'activation sont faibles comparés aux effets du temps d'adsorption, du pH, de la température et de l'hirudine.

L'activité du facteur V peut être démontrée quand ce dernier est adsorbé, mais le facteur V ne peut être élué. Le facteur X adsorbé n'a pas d'activité mais il peut être élué de la poudre de stéarate. Ceci suggère que l'activité biologique du facteur V réside dans une partie hydrophile de la molécule alors que le centre actif du facteur X est probablement masqué mais pas détruit par les surfaces hydrophobes. L'implication de ces résultats est discutée en rapport avec la théorie de formation de la prothrombinase sur les phospholipides.

Zusammenfassung

Die Reaktion zwischen Stearaten und Gerinnungsfaktoren besteht in der Adsorption der Gerinnungsfaktoren und ihrer teilweisen Aktivierung, die zur Thrombinbildung führt. Die Faktoren V und XI werden am leichtesten adsorbiert, gefolgt von Faktor X.

Die Aktivierung wird mit kleinen Mengen des Adsorbens am deutlichsten beobachtet. Sie kann durch Weglassen der Ca^{++}-Ionen oder Zugabe von Hirudin gehemmt werden. Daraus kann auf eine wahrscheinliche Beteiligung der Thrombinbildung an der Aktivierung geschlossen werden. Es konnte jedoch kein Thrombin in der Reaktionsmischung gefunden werden, da dieses sehr stark an Stearat adsorbiert wird. Die Wirkungen der Aktivierung sind unbedeutend, verglichen mit den Wirkungen der Adsorptionszeit, des pH, der Temperatur und von Hirudin.

Aktivität des Faktor V kann auch im Zustand der Adsorption nachgewiesen werden, aber der Faktor kann nicht wieder eluiert werden. Faktor X ist adsorbiert inaktiv und kann vom Pulver eluiert werden. Daraus kann geschlossen werden, daß die biologische Aktivität des Faktors V im hydrophilen Teil des Moleküls gelegen ist, während das aktive Zentrum des Faktors X höchstwahrscheinlich verborgen ist, aber nicht durch die hydrophobe Oberfläche zerstört wird. Die Bedeutung dieser Befunde hinsichtlich des Konzepts der Prothrombinasebildung an Phospholipiden wird diskutiert.

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